

COMPOSITIONS AND PROCESSES USING SIRNA, AMPHIPATHIC COMPOUNDS AND POLYCATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 This application is a Continuation-In-Part of US Application Serial No. 10/186,757 filed July 1, 2002, US Application Serial No. 10/157,674 filed May 23, 2002, and US Application Serial No. 10/345,021 filed January 15, 2003.

FIELD OF THE INVENTION

bears resemblance to RNase D, suggesting that its gene product acts in the mRNA degradation step of the reaction [Sharp 2001].

Although the use of easily manipulated model systems such as *C. elegans* and *D.*

5 *melanogaster* in gene function studies can yield clues concerning possible new drug targets in mammals, a more direct approach would be to study gene function in mammalian model systems. It has previously been demonstrated that dsRNA can be used to induce RNAi and inhibit target gene expression in mouse oocytes and early embryos [Sharp 2001; Hammond et al. 2000]. However, data obtained in a number of other studies have indicated that the use of
10 dsRNA to induce RNAi in cultured mammalian cells or post-embryonic tissue may not be effective as a sequence-specific method of gene silencing [Sharp 2001; Hammond et al. 2000]. This discrepancy may be due in large part to

and effectiveness of siRNA-mediated RNAi in cultured mammalian cell lines and suggest that the interferon response is not activated by siRNAs of this length. These results suggest that RNAi can indeed be used to effectively inhibit gene expression in mammalian cells.

5 The ability to specifically inhibit expression of a target gene by RNAi has obvious benefits. For example, many diseases arise from the abnormal expression of a particular gene or group of genes. RNAi could be used to inhibit the expression of the deleterious gene and therefore alleviate symptoms of a disease or even provide a cure. For example, genes contributing to a cancerous state or to viral replication could be inhibited. In addition, mutant genes causing
10 dominant genetic diseases such as myotonic dystrophy could be inhibited. Inflammatory diseases such as arthritis could also be treated by inhibiting such genes as cyclooxygenase or cytokines. Examples of targeted organs

trimethylammonium chloride), a number of cationic lipids have been synthesized for this purpose. Essentially all the cationic lipids are amphipathic compounds that contain a hydrophobic domain, a spacer, and positively-charged amine(s). The cationic lipids are sometimes mixed with a fusogenic lipid such as DOPE (dioleoyl phosphatidyl ethanolamine) to form liposomes. The cationic liposomes are then mixed with plasmid DNA and the binary complex of the DNA and liposomes are applied to cells in a tissue culture dish or injected *in vivo*. The ease of mixing the plasmid DNA with the cationic liposome formulation, the ability of the cationic lipids to complex with DNA and the relative high levels of transfection efficiency has led to increasing use of these formulations. However, these

together significantly increased siRNA transfer efficiency. The siRNA then inhibits expression of a selected target gene.

5 In a preferred embodiment, the polycation is a polymer such as poly-L-lysine, polyvinylamine, polyethylenimine (PEI), polysilazane, polydihydroimidazolenium, polyallylamine and the like. A preferred cationic polymer is ethoxylated polyvinylamine (pVA).

10 In a preferred embodiment the polycation is a DNA-binding protein. A preferred

from an animal. These include, but are not limited to, primary liver cells and primary muscle cells and the like. The cells within the tissue are separated by mincing and digestion with enzymes such as trypsin or collagenases which destroy the extracellular matrix. Tissues consist of several different cell types and purification methods such as gradient centrifugation or antibody sorting can be used to obtain purified amounts of the preferred cell type. For example, primary myoblasts are separated from contaminating fibroblasts using Percoll (Sigma) gradient centrifugation.

In another preferred embodiment, the cell can be an animal cell that is within the tissue *in situ* or *in vivo*

animal cells with minimal cellular toxicity. The combination of polycation and amphipathic compounds enhances the efficiency of siRNA delivery.

In a preferred embodiment, the present invention provides a process for delivering a siRNA to an animal cell comprising; preparing a ternary complex comprising mixing a compound of structure #1 with a siRNA and a polycation in a solution, associating the complex with an animal cell, and delivering the siRNA to the interior of the cell. The siRNA then inhibits expression of a gene in the cell. The amphipathic compound may be mixed with the polycation

FIG. 2. Illustration of the chemical structure for Polyimidazolinium polymer imidazolinium subunits. A) Imidazolinium Subunit, B) 2-Imidazoline Subunit.

5 FIG. 3. Illustration of the chemical structure for: A) MC7

DETAILED DESCRIPTION

The present invention describes cationic amphipathic compounds, and the methods of preparation thereof, that enhance delivery of a siRNA to an animal cell wherein the compounds have

The compositions are compatible with multi-well formats for delivery of siRNA to cells *in vitro*. Multiwell formats include cell culture plates that contain multiple wells for growing cells. Multiwell plates can be selected from the group comprising: 6-well plates, 12-well plates, 24-well plates, 96-well plates, and 384-well plates 864-well plates, and 1536-well plates. The described compositions can be dried in multiwell plates with or without siRNA. Such plates would allow high throughput screening using siRNA. Such plates could

queosine, 2-thiocyto-sine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocyto-sine, and 2,6-diaminopurine. Nucleotides are the monomeric units of nucleic acid polymers and are linked together through the phosphate groups in natural pol

be less proliferative or cancerous (e.g., less metastatic), or interfere with the replication of a virus. Intracellular proteins can be part of the cytoskeleton (e.g., actin, dystrophin, myosins, sarcoglycans, dystroglycans) and thus have a therapeutic effect in cardiomyopathies and musculoskeletal diseases (e.g., Duchenne's muscular dystrophy, limb-girdle disease). Other therapeutic proteins of particular interest to treating heart disease include polypeptides affecting cardiac contractility (e.g., calcium and sodium channels), inhibitors of

Other Definitions:

Lipid -

Any of a diverse group of organic compounds that are insoluble in water, but soluble in organic solvents such as chloroform and benzene. Lipids contain both hydrophobic and hydrophilic sections. Lipids is meant

Phospholipids -

Phospholipids are lipids having both a phosphate group and one or more fatty acids (as esters of the fatty acid). The phosphate group may be bound to one or more additional organic groups.

In a 2-imidazoline (imidazoline subunit), substituents R1, R2, R4a, R4b, R5a, and R5b can independently be a hydrogen radical or a carbon radical with any substitution.

fumaric acid, tartaric acid, citric acid, benzoic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenes

Nuclear localizing signals enhance the targeting of the pharmaceutical into proximity of the nucleus and/or its entry into the nucleus. Such nuclear transport signals can be a protein

Interaction Modifiers -

